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CONFIRMATION OF DIPLOID SPERM IN HUMAN SEMEN USING MULTI-PROBE FLUORESCENCE IN SITU HYBRIDIZATION AND PHASE CONTRAST MICROSCOPY

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Diploid sperm have been implicated in the origin of some hydatiform moles and their frequencies in semen may be associated with reduced fertility. Multi-color simultaneous Florescence in situ hybridization with chromosome-specific allows rapid screening of sperm with various aneuploidy and polyploidy genotypes. Hyperploidy was distinguished from diploidy using hybridization with probes specific for chromosomes X, Y, and 8 and using a triple-band pass filter. The XY88 phenotype may represent either a somatic cell in semen or a diploid sperm which was further distinguished by inspection for sperm tails using phase contrast microscopy.

For semen samples of 13 healthy men we characterized 486 XY88, 52 XX88 and 42 YY88 cells for the presence of tails. The length and width of 52 XY88, 19 XX88 and 13 YY88 sperm were measured. Dimensions of 5 neighboring normal sperm were used for reference.

Tails were detected in 87% of normal haploid sperm and in 77% of diploid cells ($p=0.001$). The average area of diploid sperm was ~2.2 times larger then normal haploid sperms (48.3 ± 11.6 vs. 21.6 ± 8.4 μm^2). XX88 and YY88 sperm were similar in size to diploid sperm (45.5 ± 12.8 μm^2).

Our work provides direct evidence that the most of diploid cells in semen found by FISH are diploid sperm rather than somatic cells. Seven of our donors had between 0.5 and 1 % diploid sperm and the other six had fewer. Assuming an average sperm concentration of $50\text{x}10^6$/ml these donors had up to 5×10^6 diploid sperm per ml of semen. Further studies are needed to determine whether diploid sperm have significant impact on reproductive outcome and to determine whether certain chemical exposures or physiological conditions produce more diploid sperm or somatic cells in semen.

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